Supplementary Information for

Electrospun ZnO/TiO_2 Composite Nanofibers as a Bactericidal Agent

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1. Experimental section

Preparation of precursor solution for electrospinning

The 8 wt% of poly(methyl methacrylate) (PMMA) (MW= 350,000, Aldrich) and the 10 wt% of titanium isopropoxide (TTIP) (97%, Aldrich) were dissolved in the mixed solution of dimethylformamide (DMF)/acetic acid (5:1, v:v, Aldrich) at 60 °C for 6 h with magnetic stirring. Then 5 mL of DMF solution containing 8 wt% of PMMA and 10 wt% of zinc acetate was added to the 50 mL of prepared solution and vigorously stirred for 1 h. In addition, the precursor solution for pristine TiO_2 nanofibers are prepared *via* the mixing of PMMA, DMF/acetic acid(5:1), and TTIP. The obtained precursor solution was used as the working fluid for electrospinning.

Fabrication of ZnO/TiO_2 nanofibers

The 10 mL of the precursor solution was loaded into a 25 mL syringe with an 18-gauge blunt tip needle. The flow rate of solution was 6 μL/min controlled by syringe pump purchased from Nano NC Co. (Korea). A voltage of 10 kV was applied between the needle and the copper foil, which was grounded and used to collect the nanofibers. After then, the electrospun nanofibers were calcined at 500 °C for 3 h.

Characterization

Images of transmission electron microscopy (TEM) were obtained with a JEOL JEM-200CX. Acceleration voltage for TEM was 200 kV. The images of field emission scanning electron microscopy (FE-SEM) were obtained using a JEOL 6700 at an acceleration voltage of 10 kV and energy dispersion spectroscopy (EDS) was taken with INCA energy (Oxford Instrument Analytical Ltd.) The structure of ZnO/TiO_2 nanofibers were characterized by high-powder X-ray diffraction (XRD) (M18XHF-SRA (Mac Science Co.) with a Cu Kα radiation source (λ= 1.5406 Å) at 40 kV and 300 mA (12 kW). The X-ray photoelectron spectroscope(XPS) was determined by ASCA Lab220i-XL electron spectrometer from VG Scientific using 300 W Al Kα radiation and base pressure of 3×10^{-6} bar.

Antimicrobial performance investigation

The microorganisms were cultivated in sterilized LB broth and then incubated overnight at 37 °C with a shaking incubator. The 1 mL of bacterial suspensions employed for the antibacterial experiments contained from 10^6 to 10^7 colony forming units (CFU). For investigation of bactericidal performances of the fabricated ZnO/TiO_2 nanofibers under dark condition, at first, the samples were dispersed in distilled water (1 mg/mL) with sonication for 30 min. The TiO_2 nanofibers dispersed water was prepared as a comparison material. Then, the 1 mL of each suspension was putted in sterilized test tubes and inoculated with the 100 µL of *E. coli* and *S. aureus* suspension. The bacteria inoculated solutions were incubated in shaking incubator in absence of light. In each tube, the 50 µL of aliquots were taken as a function of contact time (from 0 min to 60 min) and cultured in LB agar plates. Then, the LB agar plates were kept at 37 °C for 24 h, and the number of grown bacterial colonies was observed and counted.

In order to investigate the comparative antimicrobial activities under UV irradiation, TiO_2 nanofibers and ZnO/TiO_2 nanofibers were dispersed in methanol solvent (1 mg/mL) and coated onto polystyrene Petri dishes. The coated Petri dishes were dried in a drying oven at 70 °C for the complete evaporation of methanol solvent. As a control, only methanol-treated Petri dishes were also prepared. Diluted *S. aureus* solution (10^4 CFU/mL) was sprayed onto the Petri dish and half of the dish was wrapped in black tape to protect the half from the UV irradiation. After air-drying for 5 min, the Petri dish was set under the UV light of 312 nm (6W) for 30 second. Then, the autoclaved growth bacterial medium (which was cooled to *ca*. 40 °C) was added into the Petri dishes and solidified. The test dishes were incubated at 37 °C for 24 h and the bacterial colonies were inspected. Similarly, the TiO_2 nanofibers and control surfaces were also applied to the antimicrobial abilities with and without UV light.

2. Antibacterial activity of pure ZnO nanofibers under dark conditions

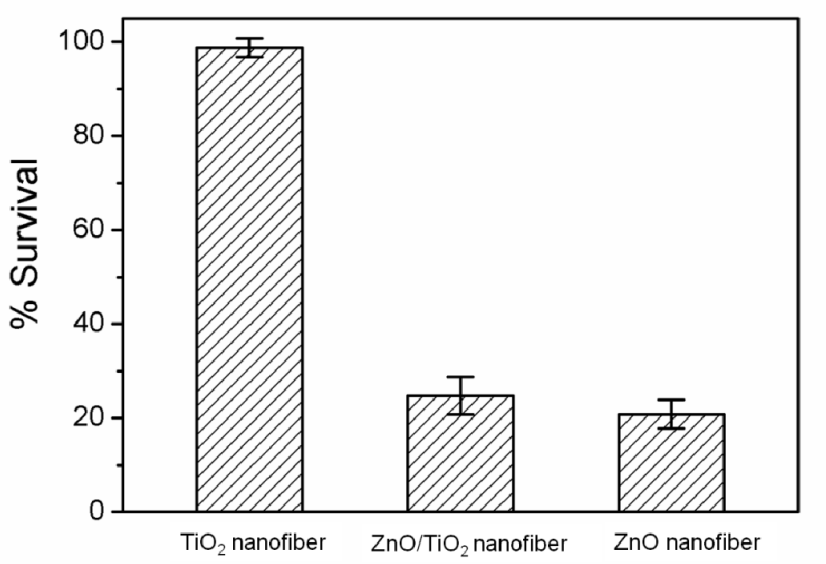

Fig. S1. Antibacterial properties of the TiO_2 nanofiber, ZnO/TiO_2 nanofiber, and ZnO nanofiber against *S. aureus* under dark condition. The % Survival was calculated as % Survival = (A/B) × 100 (where A is the number of surviving bacterial colonies of the test sample and B is that of the control)

Additional experiments about the antibacterial properties of pure ZnO nanofibers were performed. The pristine ZnO nanofibers were fabricated by the electrospinning method under similar condition to the ZnO/TiO_2 composite nanofiber. The ZnO/TiO_2 composite nanofibers, pure TiO_2 nanofibers, and pure ZnO nanofibers were prepared for antibacterial test under dark condition. Each 1 mg of sample was inoculated with *S. aureus* (10^5 – 10^6 CFU) and incubated for 10 min at 37 °C shaker. Then, in each sample, the aliquots were taken and cultured in LB agar plates. The LB agar plates were kept at 37 °C for overnight, and the number of grown bacterial colonies was observed and counted. As a result, the pristine ZnO nanofiber showed the enhanced antibacterial performance compared to the ZnO/TiO_2 nanofiber. The pure ZnO nanofiber exhibited slightly enhanced antibacterial activity (*ca.* 80.2 %), while the composite nanofiber exhibited about 75.3 % of inhibition efficiency under dark condition. Compared with the ZnO/TiO_2 composite nanofiber, the pure ZnO nanofiber has more ZnO clusters on its surface because the ZnO nanofiber consists of only ZnO cluster. Therefore, the pristine ZnO nanofiber can react with water more times and form more

hydroxyl radicals than the composite nanofibers, leading the slightly enhanced antibacterial activity.